HMG-CoA reductase inhibitor improves endothelial dysfunction in mineralocorticoid hypertension by inhibition of RhoA

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Abstract: Statins reduce cardiovascular morbidity and mortality. These beneficial effects are not fully explained by their lipid-lowering action. As such, we investigated the impact of a new statin, rosuvastatin, on endothelial function, the key event in early atherogenesis, in an experimental model of normocholesterolemic hypertension. Hypertension was induced in Wistar-Kyoto rats by inhibition of 11 -hydroxysteroid dehydrogenase type 2 (11 -HSD2) with Glycyrrhizic acid (GA). 11 -HSD2 provides mineralocorticoid receptor specificity for aldosterone by metabolising glucocorticoids to their receptor inactive 11-dehydro derivatives. GA was added to the drinking water (3 g/L) for 21 days. From days 8 to 21 rosuvastatin (20 mg/kg/d) or placebo were added to chow. Endothelium-dependent and -independent relaxation of isolated aortic rings to acetylcholine (ACh, 10-10-10-5 mol/L) and sodium nitroprusside (SNP, 10-10-10-5 mol/L) was measured. In addition, vascular reactivity to endothelin-1 (ET-1; 10-10-10-7 mol/L) was investigated. ETA and ETB receptor mRNA expression was determined by RT-PCR and RhoA activity by a pull-down assay. Systolic blood pressure increased in rats treated with GA (175 vs 153 mmHg in controls; $p<0.01$). Endothelium-dependent relaxations to acetylcholine were blunted after GA treatment (p 0.005 vs control), while the responses to SNP remained unchanged. Rosuvastatin normalized NO-mediated endothelium-dependent relaxation in hypertensive animals (p 0.01 vs placebo), although blood pressure and cholesterol levels were not affected by the statin. Vascular reactivity to ET-1 was increased by GA ($p<0.01$ vs control), but not affected by rosuvastatin. ETB receptor mRNA decreased in the GA group ($p<0.05$ vs control) and was upregulated by rosuvastatin ($p<0.005$; GA+rosuvastatin vs GA), whereas ETA receptor mRNA upregulation in the GA group ($p<0.01$ vs control) was partially prevented by the statin. In addition, GA increased Rho-GTP binding (p 0.05 vs control) which was prevented in both groups by rosuvastatin treatment (p 0.01 control+rosuvastatin vs control and GA+rosuvastatin vs GA). These data for the first time show that HMG-CoA inhibition improves endothelial dysfunction in normocholesterolemic mineralocorticoid hypertension without affecting blood pressure or cholesterol levels by correction of a stimulated endothelin system

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	Control	Ang II (n = 8)	Ang II-AML (n = 7)
SBP (mmHg)	135 ± 2	175 ± 3*	132 ± 6
Body Weight (g)	288 ± 10	261 ± 11**	261 ± 10**
Ao weight (mg/cm)	13.2 ± 0.3	16.9 ± 1.3*	13.4 ± 0.4
O_2^- (cpm/mg/min)	608 ± 159	1005 ± 140*	595 ± 62
$ONOO^-$ (cpm/mg/min)	782 ± 115	1875 ± 295*	1142 ± 134
Emax (% of NE contraction)	105 ± 4	86 ± 3*	102 ± 3
ED50 (− Log M)	8.0 ± 0.27	6.6 ± 0.19*	7.6 ± 0.18

** $P < 0.05$ vs Control and Ang II—AML; ** $P < 0.05$ vs Control*

beneficial synergistic effects upon the blood pressure as well as the vasculature.

Key Words: Angiotensin II, Reactive Oxygen Species, Nitric Oxide

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HMG-COA REDUCTASE INHIBITOR IMPROVES ENDOTHELIAL DYSFUNCTION IN MINERALOCORTICOID HYPERTENSION BY INHIBITION OF RHOA

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Statins reduce cardiovascular morbidity and mortality. These beneficial effects are not fully explained by their lipid-lowering action. As such, we investigated the impact of a new statin, rosuvastatin, on endothelial function, the key event in early atherogenesis, in an experimental model of normocholesterolemic hypertension.

Hypertension was induced in Wistar-Kyoto rats by inhibition of 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) with Glycyrrhizic acid (GA). 11β-HSD2 provides mineralocorticoid receptor specificity for aldosterone by metabolising glucocorticoids to their receptor inactive 11-dehydro derivatives. GA was added to the drinking water (3 g/L) for 21 days. From days 8 to 21 rosuvastatin (20 mg/kg/d) or placebo were added to chow. Endothelium-dependent and -independent relaxation of isolated aortic rings to acetylcholine (ACh, 10^{-10}-10^{-5} mol/L) and sodium nitroprusside (SNP, 10^{-10}-10^{-5} mol/L) was measured. In addition, vascular reactivity to endothelin-1 (ET-1; 10^{-10}-10^{-7} mol/L) was investigated. ETA and ETB receptor mRNA expression was determined by RT-PCR and RhoA activity by a pull-down assay. Systolic blood pressure increased in rats treated with GA (175 vs 153 mmHg in controls; $p<0.01$). Endothelium-dependent relaxations to acetylcholine were blunted after GA treatment ($p\leq0.005$ vs control), while the responses to SNP remained unchanged. Rosuvastatin normalized NO-mediated endothelium-dependent relaxation in hypertensive animals ($p\leq0.01$ vs placebo), although blood pressure and cholesterol levels were not affected by the statin. Vascular reactivity to ET-1 was increased by GA ($p<0.01$ vs control), but not affected by rosuvastatin. ETB receptor mRNA decreased in the GA group ($p<0.05$ vs control) and was upregulated by rosuvastatin ($p<0.005$; GA+rosuvastatin vs GA), whereas ETA receptor mRNA upregulation in the GA group ($p<0.01$ vs control) was partially prevented by the statin. In addition, GA increased Rho-GTP binding ($p\leq0.05$ vs control) which was prevented in both groups by rosuvastatin treatment ($p\leq0.01$ control+rosuvastatin vs control and GA+rosuvastatin vs GA).

These data for the first time show that HMG-CoA inhibition improves endothelial dysfunction in normocholesterolemic mineralocorticoid hypertension without affecting blood pressure or cholesterol levels by correction of a stimulated endothelin system.

Key Words: HMG-CoA reductase inhibitor, hypertension, endothelin

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PLASMA IMMUNOREACTIVE ENDOTHELIN-1 LEVELS IN HYPERTENSIVE RATS AND HUMAN SUBJECTS

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Endothelin-1 (ET-1) is an endothelium-derived potent vasoconstrictor peptide of 21 amino acids. To establish reference values in different forms and models of hypertension and in human subjects an assay for plasma ET-1 was optimized. Immunoreactive (ir-) ET-1 is extracted by acetone from 1 ml plasma and subjected to a sensitive sandwich type enzyme linked immunosorbent assay. The detection limit for plasma ET-1 (3 SD above zero readings) is 0.05 fmol/ml. Mean recoveries of the 1, 2, 5, 10 fmol of ET-1 added to 1 ml plasma (n = 5, each) were 66, 75, 85 and 92 % respectively. The within-assay coefficients of variation were 12, 5, 3, 3 and 0.5 % for plasma ET-1 concentrations of 0.84, 1.5, 2.3, 5.2 and 9.9 fmol/ml respectively. Between-assay coefficients of variation for two human control plasmas containing 1.0 fmol/ml (n = 8) and 1.2 fmol/ml ET-1 (n = 7) were 8% and 10% respectively. Assay accuracy was demonstrated by the consistent recoveries of added ET-1 and by the linearity of ir-ET-1 concentrations measured in serially diluted plasma extracts (r = 0.99). No ir-ET-1 was detected when albumin buffer was extracted instead of plasma (buffer blank). Using this method, we found increased ir-ET-1 levels in plasma of three experimental rat models of hypertension. (i) Plasma ir-ET-1 concentrations were significantly higher in stroke-prone spontaneously hypertensive rats (SP-SHR) than in normotensive Wistar rats. (ii) DOCA-salt hypertensive rats exhibited 4 times higher ir-ET-1 levels than sham operated control rats. (iii) One kidney-one clip (1K-1C) hypertensive rats showed moderately increased ir-ET-1 levels compared to sham operated controls. In contrast, the ir-ET-1 levels in plasma of SHR were half that of normotensive Wistar rats. In two kidney-one clip (2K-1C) Goldblatt hypertensive rats, the plasma ir-ET-1 concentrations were not different from sham-operated control rats. The plasma ir-ET-1 concentrations of 37 healthy human subjects were 0.85 ± 0.26 fmol/ml (mean ± SD). We conclude that the present assay reliably measures plasma immunoreactive ET-1 levels in rats and in human subjects. Normal plasma ET-1 concentrations in humans and conscious rats are in the low picomolar range.

Key Words: Endothelium, Vasoconstrictor, Hormone

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IDENTIFICATION OF DOMINANT NEGATIVE RAT RAMPs ABLE TO INHIBIT ENDOGENOUS ADRENOMEDULLIN RECEPTOR FUNCTION

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Adrenomedullin (AM) exerts a wide variety of biological effects, including potent vasorelaxation. Its receptors were recently shown to be heterodimers comprised of a novel accessory protein, the receptor activity-modifying protein (RAMP), and the calcitonin receptor-like receptor (CRLR). When CRLR is co-transfected with RAMP2 or -3, the two proteins are transported together to the plasma membrane where they function as an AM-specific receptor. Recently, we have shown that seven amino acids of human (h)RAMP2 (86-92) and hRAMP3 (59-65) are essential for high-affinity agonist binding to hAM receptors. Interestingly, both seven-residue segments are located between three conserved residues (Trp, Cys and Tyr) and the three ones are common to humans, rats and mice. In this study, we examined whether seven-residue segments situated between three residues conserved in both rat (r)RAMP2 and rRAMP3 (amino acids 93-99 and 58-64, respectively) are key determinants of agonist binding to rAM receptors, and then tested whether their deletion mutants can act as dominant negative RAMPs.